Transformation Protocol 2

Preparation:

All manipulations were carried out on ice. For each pulsing experiment, 30 ml of a late-exponential phase culture (OD565=1, equivalent to about 10^10 cells) was harvested by centrifugation (14,000×g for 10 min). The cells were washed twice using an equal volume of electroporation buffer (1 mM HEPES, pH 7.0, 1 mM MgCl2, 200 mM sucrose) and resuspended to a final volume of 400 µl using the same buffer.

Electroporation:

An appropriate amount (0.2–5 µg) of plasmid DNA was added. After incubation for 5 min on ice, the mixture was loaded in a chilled 0.2-cm cuvette and subjected to a single pulse using a Gene pulser and a pulse controller apparatus (Biorad, Munich; settings: 1.2 kV, 600 Ohm, 25 µF). Pre-warmed liquid growth medium (4.5 ml) was added immediately, and the cells were incubated for 3 h at 28 °C for regeneration before they were plated onto ACA medium supplemented with the appropriate antibiotic.